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*Relationships between Antiseptic Action and Chemical Constitution with special reference to Compounds of the Pyridine, Quinoline, Acridine and Phenazine Series.**

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Biological Section.

In the course of investigations on the antiseptic properties of certain basic benzene derivatives, it was shown that the methochloride of diaminoacridine was very highly antiseptic, and that, unlike other powerful antiseptics then known, its antibacterial activity was not reduced by the presence of blood serum (Browning, Gilmour, Gulbransen, Kennaway and Thornton, 2, 7). Diaminoacridine methochloride had been prepared by

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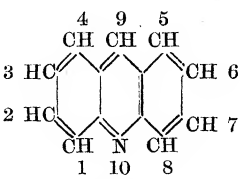
Benda (1) for Ehrlich, and was named by them trypaflavin on account of its powerful therapeutic properties in experimental trypanosome infections. We are not aware that its action on bacteria had been investigated prior to the work recorded in the above paper (2), although Shiga (10) published almost simultaneously results of his investigations on its action on *V. choleræ*. The hydrochloride and sulphate of diaminoacridine were then found to be practically equal to the methochloride in antiseptic properties and in efficacy of action in serum (8). In the case of these aminoacridine substances the power of killing bacteria is exerted slowly; thus, with the methochloride acting on *B. coli* in serum, the concentration which proves lethal in 2 hours (1 : 20000) is five to ten times greater than that required to produce sterility after 24 hours; on the other hand, mercuric chloride and carbolic acid produce their maximum effect within 2 hours, whether they be tested in a solution containing a minute amount of protein (0·7 per cent. of Witte's peptone) or in a rich protein medium such as serum. The acridine compounds, therefore, may be said to act especially as bacteriostatic agents (7, 8). This property, coupled with their relative innocuousness for mammalian tissues, as tested by toxicity for the living animal as a whole (4, 7), effect on phagocytosis (3, 7), and slight irritating action on the conjunctiva (7), suggested that these substances should be specially applicable for the purpose of restraining bacterial infections in the tissues. Thus the methochloride, under the name of acriflavine, and the sulphate of diaminoacridine base, as proflavine, found extensive use in the treatment of infected wounds during the late war, and also in the treatment of such relatively accessible infections as gonorrhœa. The present work records the investigations which have been made with the view of tracing the source of the antiseptic property of diaminoacridine compounds by examining substances which may be regarded as fragments of the acridine molecule. The parent substance of the acridine derivatives is a compound of the following formula :—



Acridine.

that is, a combination of two benzene and a pyridine ring. If the two side wings are removed, a pyridine nucleus remains; if only one wing is detached, a quinoline nucleus results. It seemed, therefore, possible that the antiseptic activity of acriflavine (diamino acridine methochloride) might reside either

in the pyridine or quinoline nucleus, reinforced by one or more amino groups. It is for this reason that substances of this type were first examined.

In addition, a series of acridine derivatives have been prepared and tested for their antiseptic power in order to determine, if possible, whether any law could be established relating chemical structure and antiseptic action within the group. Also, observations have been made upon phenazine compounds, on account of their close chemical relationship to the acridine group.

Methods of Estimating Antiseptic Power.

The substance to be tested, in a volume not exceeding 0·1 c.c., was added to 1 c.c. of the culture medium, which consisted: (*a*) of a watery solution containing 0·35 per cent. sodium chloride, and 0·7 per cent. bacteriological peptone, such as Witte's, the hydrogen-ion concentration of the mixture being adjusted by the addition of caustic soda to yield a P_H value between 7·2 and 7·8 as indicated by the usual indicators; and (*b*) sterile ox serum, which had been previously heated for several hours at 56° C., in order to destroy normal bactericidal power as well as accidental contaminating organisms. Serum, in virtue of its content in protein, has a powerful action in reducing the bactericidal effect of most strong antiseptics, at the same time it represents the fluid constituent to which antiseptics in contact with the tissues are exposed, *e.g.*, in a surgically treated wound; serum also acts as a satisfactory culture medium for the two types of organisms employed, and is extremely constant in composition and reaction. Hence, serum may be regarded as a highly suitable medium in which to test antiseptic action. The organisms employed in the tests were *Staphylococcus aureus* and a single strain of *B. coli*; the inoculation dose being 0·1 c.c. of a 1 : 1000 dilution in saline of a 24 hours' culture in peptone water. Experiments have shown that within wide limits the efficiency of the antiseptic, as tested by the method described, is practically independent of the size of the inoculum (3, 8). But inoculation with very large numbers of organisms should be avoided, as these fail to maintain themselves in the medium even in the absence of any antiseptic (3). In general, the following series of concentrations of each substance was tested, 1 : 1000000, 1 : 400000, 1 : 200000, 1 : 100000, 1 : 40000, 1 : 20000, 1 : 10000, 1 : 4000, 1 : 2000, 1 : 1000. Thus, in examining any given compound, the effect of varying concentrations was tested at the same time and with the same batch of medium and the same cultures of organisms. The mixtures were incubated at 37° C. for 48 hours, and then the final readings were made; the occurrence of abundant growth was shown by the development of turbidity in the previously clear medium, but subcultures frequently yielded growth from tubes which appeared to be

clear to the naked eye. Accordingly, in all cases the presence or absence of living organisms was decided by subculturing each mixture on nutrient agar, which was then incubated for 48 hours at 37° C. The results are recorded numerically, the highest concentration which permitted vigorous growth, and the lowest producing sterility, as tested by subculture of a loopful of the mixture on agar, being given. In certain cases there is a wide zone separating these two concentrations, which indicates that the particular compounds are specially bacteriostatic in action, and that concentrations considerably less than that required to kill the bacteria still have the effect of restraining growth. It is to be noted that more useful information is obtained by making subcultures from the mixtures of antiseptic and organisms on a solid medium than in a fluid one, as in the latter case it is not possible to determine any degree of action of the antiseptic short of complete sterilisation. The investigation has been complicated by such questions as effects due to differences in solubility and variations in dissociation, leading possibly to differences in hydrogen-ion concentration of the solutions. Thus it has been found, when examining particular compounds, that comparatively small variations in hydrogen-ion concentration may exercise a great influence on the antiseptic potency (Browning, Gulbransen and Kennaway, 6). With diaminoacridine methochloride in peptone water, the concentration required to sterilise *B. coli* when the P_H value of the solution lay between 4 and 5 was 1 : 2000; within a range from 6 to 7, 1 : 10000 of the dye sufficed; at 8 to 9, 1 : 40000 sterilised, while at 11 a concentration of 1 : 200000 was sufficient. In each case the medium with similar reaction, but without the antiseptic, permitted vigorous growth of the organisms. In addition, there is the further factor of variability in the behaviour of the bacterial culture. With regard to the latter, it appears to be highly probable, if not definitely established, that the individuals in a given culture are not all equally susceptible to harmful influences; thus, irregularities are observed when a particular concentration of antiseptic is caused to act on duplicate samples of the same infected material. This has been drawn attention to by Richet and Cardot (9), and has been observed also in our own work. Further, the occurrence of variations in the culture from time to time can scarcely be excluded although no evidence has been obtained pointing to permanent or to regularly cyclic changes. Repeated series of tests carried out with a view to examining the action of diaminoacridine methochloride on a single strain of *B. coli* in ox serum have shown the following variable results. A concentration of 1 : 1000000 and upwards, sterilised in 8 series, 1 : 400000 and upwards, sterilised in 17 series, 1 : 200000 and upwards in 16 series,

1 : 100000 and upwards, in 24 series, 1 : 40000 in 2 series, and 1 : 40000 failed to sterilise in 1 series. With *Staphylococcus aureus*, however, the range of variation was distinctly less. Accordingly, the numerical values recorded here must be interpreted in the light of the above results (5). It should be noted, however, that in comparing certain closely related compounds in which marked alterations in antiseptic power may be produced by relatively slight chemical differences, *e.g.*, the methochloride and the hydrochloride of the same base, the two substances have been tested on the same occasion and with the same specimen of medium and culture, thereby reducing as far as possible the action of uncontrollable factors.

Fragments of the Acridine Molecule.

Table I includes all the compounds tested, and comprises pyridine and quinoline derivatives and dinaphthylimine. The striking feature, in general, is the low grade of antiseptic power shown by these bodies. Thus, the hydrochlorides of quinoline [3], tetrahydroquinoline [15], and the amino-quinolines (*o* [4], *m* [6], *p* [8], and *a* [10]), all failed to sterilise in dilutions exceeding 1 : 2000 either in peptone water or in serum. The methochlorides of the aminoquinolines [7, 9, 11], except in the case of the ortho-compound [5], showed accentuation of antiseptic action in serum, as compared with the hydrochlorides of the corresponding bases, a characteristic result which will be discussed in more detail when dealing with the acridine group. The hydrochlorides of α- [17] and β-naphthoquinoline [19] were slightly more active.* No striking difference could be established between these and their tetrahydro-derivatives [22, 23]. Diamino β-naphthoquinoline [24] also showed no enhanced efficiency. The methochlorides (or methosulphates) of both naphthoquinolines [18, 20, 21] and of diamino-β-naphthoquinoline [25] showed intensified action in serum. The 8-hydroxyquinoline sulphate [12], long known as an antiseptic under the name of "chinosol," is included for comparison; its activity for *Staphylococcus aureus* contrasts with the slight effect of hydroxyacridine compounds [67] and of the aminoquinoline compounds as antiseptics, but it is very weakly antiseptic for *B. coli*. On the other hand, it is remarkable that the methochloride [13] and methopicrate [14] of the base do not show enhanced action. 1·1·dinaphthyl-2·2·imine [28] exhibits great discrepancy between its powerful action on *staphylococcus* and lack of effect on *B. coli*, which is similar to that exhibited by the triamino-triphenylmethane compounds, hexa-methyl and ethyl-violet. But the most striking character of this substance is the reduction in action produced by

* Previous results recorded for α- and β-naphthoquinoline were obtained with less pure preparations (see 'Journal of Pathology and Bacteriology,' vol. 24, p. 127 (1921)).

Table I.—The Antiseptic Action of Fragments of the Acridine Molecule—Quinoline and Pyridine Derivatives and Dinaphthylimine.

(In this and subsequent Tables + indicates that free growth of the organisms occurred in the concentration of substances mentioned, while — indicates a sterile mixture; *inh.* denotes inhibition of growth short of complete sterilisation. *Ppt.* indicates that precipitation has occurred in the mixture of medium and chemical compound.)

No.	Substance.	Organism.			
		Staphylococcus aureus: medium		*B. coli*: medium	
		Peptone water.	Serum.	Peptone water.	Serum.
1	α-aminopyridine hydrochloride	1 : 4000 +	1 : 1000 +	1 : 1000 +	1 : 1000 +
		? —	? —	? —	? —
2	α-dimethylaminopyridine methiodide	1 : 20000 +	1 : 10000 +	1 : 1000 +	1 : 100000 +
		1 : 2000 —	? —	? —	? —
3	Quinoline hydrochloride	1 : 4000 +	1 : 1000 +	1 : 2000 +	1 : 1000 +
		1 : 2000 —	? —	1 : 1000 —	? —
4	o-aminoquinoline hydrochloride	1 : 4000 +	1 : 1000 +	1 : 1000 +	1 : 1000 +
		1 : 2000 —	? —	? —	? —
5	o-aminoquinoline methochloride	1 : 4000 +	1 : 1000 +	1 : 1000 +	1 : 1000 +
		1 : 2000 —	? —	? —	? —
6	m-aminoquinoline hydrochloride	1 : 2000 +	1 : 1000 +	1 : 1000 +	1 : 1000 +
		1 : 1000 —	? —	? —	? —
7	m-aminoquiniline methochloride	1 : 4000 +	1 : 10000 +	1 : 1000 +	1 : 10000 +
		1 : 1000 (*inh.*)	1 : 4000 —	? —	1 : 4000 —
8	p-aminoquinoline hydrochloride	1 : 1000 +	1 : 1000 +	1 : 1000 +	1 : 1000 +
		? —	? —	? —	? —
9	p-aminoquinoline methochloride	1 : 2000 +	1 : 10000 +	1 : 1000 +	1 : 40000 +
		1 : 1000 (*inh.*)	1 : 2000 —	? —	1 : 4000 —
10	α-aminoquinoline hydrochloride	1 : 4000 +	1 : 1000 +	1 : 1000 +	1 : 1000 +
		1 : 1000 (*inh.*)	? —	? —	? —
11	α-aminoquinoline methochloride	1 : 1000 +	1 : 10000 +	1 : 1000 +	1 : 10000 +
		? —	1 : 4000 —	? —	1 : 4000 —

12	8-hydroxyquinoline sulphate ("chinosol")	1 : 2000000 +	1 : 400000 +	1 : 40000 +	1 : 40000 +
		1 : 400000 −	1 : 100000 −	1 : 1000 −	1 : 1000 −
13	8-hydroxyquinoline methochloride	1 : 400000 +	1 : 20000 +	1 : 10000 +	1 : 4000 +
		1 : 100000 −	1 : 10000 −	1 : 1000 −	1 : 1000 −
14	8-hydroxyquinoline methopicrate.....	1 : 100000 +	1 : 20000 +	1 : 2000 +	1 : 4000 +
		1 : 40000 −	1 : 4000 −	1 : 1000 −	1 : 2000 −
15	Tetrahydroquinoline hydrochloride	1 : 2000 +	1 : 1000 +	1 : 1000 +	1 : 1000 +
		1 : 1000 −	? −	? −	? −
16	Methyl-tetrahydroquinoline methiodide	1 : 1000 +	1 : 1000 + (*ppt.*)	1 : 1000 +	1 : 1000 + (*ppt.*)
		? −	? −	? −	? −
17	α-naphthoquinoline hydrochloride	1 : 10000 +	1 : 2000 +	1 : 2000 + (*ppt.*)	1 : 1000 +
		1 : 4000 −	1 : 1000 (*inh.*)	1 : 1000 − (*ppt.*)	? −
18	α-naphthoquinoline methosulphate	1 : 20000 +	1 : 40000 +	1 : 4000 +	1 : 100000 +
		1 : 4000 −	1 : 10000 −	1 : 1000 −	1 : 40000 −
19	β-naphthoquinoline hydrochloride	1 : 10000 +	1 : 2000 +	1 : 10000 +	1 : 1000 +
		1 : 4000 − (*ppt.*)	1 : 1000 (*inh.*)	1 : 4000 − (*ppt.*)	? −
20	β-naphthoquinoline methochloride	1 : 20000 +	1 : 20000 +	1 : 2000 +	1 : 100000 +
		1 : 10000 −	1 : 10000 −	1 : 1000 −	1 : 10000 −
21	β-naphthoquinoline methosulphate	1 : 40000 +	1 : 20000 +	1 : 1000 +	1 : 40000 +
		1 : 4000 −	1 : 10000 −	? −	1 : 4000 −
22	Tetrahydro-α-naphthoquinoline hydrochloride	1 : 10000 + (*ppt.*)	1 : 1000 +	1 : 10000 + (*ppt.*)	1 : 1000 +
		1 : 4000 − (*ppt.*)	? −	1 : 4000 − (*ppt.*)	? −
23	Tetrahydro-β-naphthoquinoline hydrochloride	1 : 10000 +	1 : 2000 +	1 : 4000 + (*ppt.*)	1 : 1000 +
		1 : 2000 − (*ppt.*)	1 : 1000 (*inh.*)	1 : 2000 − (*ppt.*)	? −
24	Diamino-β-naphthoquinoline*	1 : 4000 +	1 : 4000 +	1 : 2000 +	1 : 1000 +
		1 : 2000 −	1 : 1000 −	1 : 1000 (*inh.*)	? −
25	Diamino-β-naphthoquinoline methochloride	1 : 2000 +	1 : 10000 +	1 : 1000 +	1 : 10000 +
		1 : 1000 −	1 : 4000 −	? −	1 : 4000 −
26	1.4.naphtho-dipyridine hydrochloride†	1 : 4000 + (*ppt.*)	1 : 1000 + (*ppt.*)	1 : 4000 + (*ppt.*)	1 : 1000 + (*ppt.*)
		1 : 1000 − (*ppt.*)	? −	1 : 2000 − (*ppt.*)	? −
27	1.5.naphtho-dipyridine hydrochloride†	1 : 2000 +	1 : 1000 + (*ppt.*)	1 : 2000 +	1 : 1000 + (*ppt.*)
		1 : 1000 −	? −	? −	? −
28	1.1.dinaphthyl-2.2.imine	? +	1 : 1000 +	1 : 1000 + (*ppt.*)	1 : 4000 +
		1 : 2000000 −	? −	? −	? −

* This compound dissolved in water without the addition of acid.

† 1 : 1000 solution strongly acid.

serum; thus 1 : 2000000 sterilised staphylococci in watery medium, but 1 : 1000 failed to kill these organisms in serum. This is the most extreme reduction observed in the case of any substance, being twenty times greater than the reduction effected by serum on mercuric chloride.

So far, therefore, it has not been possible to obtain any fragment of the molecule which equals, or even approximates closely to, diaminoacridine in antiseptic properties.

Acridine Group.

The substances which have been tested are included in Table II. The following general conclusions may be drawn from the results :—

Action of the Amino-Groups.—The introduction of amino-groups enhances the antiseptic potency both for *Staphylococcus aureus* and *B. coli*, *e.g.*, acridine [29] and diaminoacridine [35], dimethylacridine [33] and diaminodimethylacridine [48].

Effectiveness in Serum.—Effectiveness in serum is a characteristic of the compounds with unsubstituted amino-groups and especially of the methochlorides of these bases. The further introduction into the diamino compounds of a phenyl-group attached to the medial carbon atom (in position 9) has, however, a marked effect in diminishing the action in serum ; this is exhibited both in the case of 2·7·diamino-3·6·dimethylacridine [48—51] and 2·amino-3·methylnaphthacridine [58—61]. On the other hand, the methochloride of 9·phenylacridine [32] is more active than that of acridine [30].

Comparison of the Antiseptic Power of the Methochloride and the Hydrochloride of the same Base.—The methochloride (or methosulphate or methonitrate) is never less potent than the hydrochloride in the presence of serum and in some cases the increased effectiveness shown by the methochloride is very remarkable, *e.g.*, 2·7·tetraethyldiaminoacridine [55, 56], 2·7·diamino-3·6·dimethyl-9·phenylacridine [50, 51], 2·amino-3·methylnaphthacridine [58, 59], 2·dimethylaminonaphthacridine [62, 63]. In the case of the simplest member of the amino series, 2·7·diaminoacridine [35, 36],* and where the substituents are directly attached to the outer rings as in 2·7·diamino-3·6·dimethylacridine [48, 49], the hydrochloride and the methochloride are practically equal in antiseptic power. It is noteworthy, however, that when the antiseptic power of diaminoacridine is diminished by substitution of ethyl radicals in the amino-groups, the enhanced action of the methochloride [56] over the hydrochloride [55] again becomes apparent. So far, no rational explanation of the enhanced efficacy of the methochloride has suggested itself.†

* This result has been obtained with carefully purified specimens of these compounds.

† The observations of Crum Brown and Fraser on the change produced in the pharmacological action of alkaloids when a methyl group is attached to a nitrogen

The hydrochlorides of certain of the compounds require the presence of a slight excess of hydrochloric acid in order to effect solution, *e.g.*, in the case of 9.phenylacridine [31] and 2.amino-3.methylnaphthacridine [58]; but the enhanced effect of the methochlorides [32, 59] over the respective hydrochlorides [31, 58] is not to be ascribed to the higher hydrogen-ion concentration of the solution of the latter, since the addition of hydrochloric acid to the methochloride, so as to produce a solution of similar reaction, did not reduce the antiseptic power to that of the hydrochloride. The comparative effects of the hydrochloride and the methochloride of the same base in peptone water show a much less regular behaviour.

The Substitution of other Radicals for the Methyl Group in Diaminoacridine Methochloride.—The following were examined : ethyl [37], propyl [38], *n*- [39] and iso-butyl [40], iso-amyl [41], phenyl [42], benzyl [43], also the chloroacetate [44], chloropropionate [45], and chloroacetanilide [46] derivatives. The result was that, within the limits of experimental variation, these compounds are practically identical with the methochloride [36] in their antiseptic power for both organisms.

The Substitution of Alkyls in the Amino Groups.—Tetramethyl- [52] and tetraethyl-diaminoacridine [55] hydrochloride and also the methochlorides [53, 56] and methonitrate [54, 57] were investigated. The tetramethyl hydrochloride [52], while practically equal to unsubstituted diaminoacridine [35] in its action on *Staphylococcus aureus*, was distinctly inferior for *B. coli*, both in peptone water and in serum. The tetraethyl compound [55] was still weaker; thus with the latter the sterilising concentration for staphylococcus in peptone water was 1 : 100000 and in serum 1 : 10000, while for *B. coli* a concentration not less than 1 : 1000 was required. The methochloride [53] and methonitrate [54] of the tetramethyl compound were practically equal to the hydrochloride; also, as in the case of the unsubstituted diaminoacridine, the effect in serum with the hydrochloride and methochloride was practically equal. On the other hand, the methochloride [56] and methonitrate [57] of the tetraethyl compound were much more active in serum than the hydrochloride.

Groups which interfere with Antiseptic Action.—As has been shown above, the introduction of methyl and ethyl groups into the amino radicals depresses rather than enhances the antiseptic potency [52, 55, as compared with 35], thus contrasting with the effect of similar substituents in the diamino- and

atom, thus converting the compound into a quaternary base, should be recalled in this connection ('Trans. Roy. Soc.,' Edinburgh, vol. 25, pp. 151, 693 (1868–69); 'Proc. Roy. Soc.,' Edinburgh, vol. 6, p. 556 (1868–69)). It has been shown, however, by Lenz that diaminoacridine methochloride is totally devoid of curare action, either in cold or warm blooded animals ('Zeitschr. f. d. gesamt. experim. Med.,' vol. 12, p. 195 (1921)).

Table II.—Antiseptic Action of Acridine Derivatives.

No.	Substance.	Organism.			
		Staphylococcus aureus : medium		*B. coli* : medium	
		Peptone water.	Serum.	Peptone water.	Serum.
29	Acridine hydrochloride	1 : 20000 +	1 : 4000 +	1 : 2000 +	1 : 1000 +
		1 : 2000 —	? —	1 : 1000 —	? —
30	Acridine methochloride	1 : 10000 +	1 : 10000 +	1 : 10000 +	1 : 20000 +
		1 : 1000 —	1 : 2000 —	1 : 1000 —	1 : 4000 —
31	9.phenylacridine hydrochloride	1 : 2000 +	1 : 1000 +	1 : 2000 +	1 : 1000 +
		1 : 1000 —	? —	1 : 1000 —	? —
32	9.phenylacridine methochloride	1 : 1000000 +	1 : 200000 +	1 : 4000 +	1 : 20000 +
		1 : 40000 —	1 : 20000 —	1 : 2000 —	1 : 10000 —
33	3.6.dimethylacridine hydrochloride*	1 : 1000 + (*ppt.*)	1 : 1000 + (*ppt.*)	1 : 1000 + (*ppt.*)	1 : 1000 + (*ppt.*)
		? —	? —	? —	? —
34	3.6.dimethylacridine methochloride	1 : 40000 (*inh.*)	1 : 40000 +	1 : 10000 +	1 : 10000 +
		1 : 20000 —	1 : 4000 —	1 : 4000 —	1 : 4000 —
35	2.7.diaminoacridine hydrochloride (and sulphate)	1 : 400000 +	1 : 400000 +	1 : 40000 +	1 : 200000 +
		1 : 200000 —	1 : 200000 —	1 : 20000 —	1 : 100000 —
36	2.7.diaminoacridine methochloride	1 : 400000 +	1 : 400000 +	1 : 40000 +	1 : 200000 +
		1 : 200000 —	1 : 200000 —	1 : 20000 —	1 : 100000 —
37	2.7.diaminoacridine ethochloride	1 : 200000 +	1 : 400000 +	1 : 100000 +	1 : 400000 +
		1 : 40000 —	1 : 200000 —	1 : 10000 —	1 : 100000 —
38	2.7.diaminoacridine propylchloride	1 : 100000 +	1 : 400000 +	1 : 40000 +	1 : 400000 +
		1 : 40000 —	1 : 200000 —	1 : 10000 —	1 : 200000 —
39	2.7.diaminoacridine *n*-butylchloride	1 : 100000 +	1 : 400000 +	1 : 40000 +	1 : 400000 +
		1 : 40000 —	1 : 200000 —	1 : 10000 —	1 : 200000 —
40	2.7.diaminoacridine iso-butylchloride	1 : 20000 +	1 : 400000 +	1 : 100000 +	1 : 400000 +
		1 : 10000 —	1 : 200000 —	1 : 40000 —	1 : 200000 —
41	2.7.diaminoacridine iso-amylchloride	1 : 400000 +	1 : 400000 +	1 : 100000 +	1 : 400000 +
		1 : 100000 —	1 : 200000 —	1 : 10000 —	1 : 200000 —
42	2.7.diaminoacridine phenylchloride	1 : 200000 +	1 : 400000 +	1 : 100000 +	1 : 400000 +
		1 : 40000 —	1 : 100000 —	1 : 10000 —	1 : 100000 —
43	2.7.diaminoacridine benzylchloride	1 : 100000 +	1 : 400000 +	1 : 100000 +	1 : 400000 +
		1 : 40000 —	1 : 100000 —	1 : 20000 —	1 : 200000 —
44	2.7.diaminoacridine chloroacetate	1 : 200000 +	1 : 1000000 +	1 : 40000 +	1 : 1000000 +
		1 : 100000 —	1 : 200000 —	1 : 20000 —	1 : 400000 —
45	2.7.diaminoacridine chloropropionate	1 : 400000 +	1 : 1000000 +	1 : 200000 +	1 : 1000000 +
		1 : 200000 —	1 : 400000 —	1 : 40000 —	1 : 200000 —
46	2.7.diaminoacridine chloracetanilide	1 : 200000 +	1 : 1000000 +	1 : 20000 +	1 : 400000 +
		1 : 40000 —	1 : 200000 —	1 : 10000 —	1 : 100000 —

47	2.7.diaminoacridine methochloride + C_2H_5I	1 : 200000 +	1 : 1000000 +	1 : 100000 +	1 : 200000 +
		1 : 100000 —	1 : 100000 —	1 : 40000 —	1 : 40000 —
48	2.7.diamino-3.6.dimethyl-acridine hydrochloride (acridine yellow)	1 : 400000 +	1 : 400000 +	1 : 1000 +	1 : 200000 +
		1 : 20000 —	1 : 100000 —†	? —	1 : 100000 —
49	2.7.diamino-3.6.dimethyl-acridine methochloride...	1 : 1000000 +	1 : 400000 +	1 : 40000 +	1 : 400000 +
		1 : 400000 —	1 : 200000 —	1 : 10000 —	1 : 100000 —
50	2.7.diamino-3.6.dimethyl-9.phenylacridine hydrochloride (benzoflavine)	1 : 400000 +	1 : 20000 +	1 : 1000 +	1 : 1000 +
		1 : 200000 —	1 : 10000 —	? —	? —
51	2.7.diamino-3.6.dimethyl-9.phenylacridine methochloride	1 : 4000000 +	1 : 1000000 +	1 : 4000 +	1 : 40000 +
		1 : 2000000 —	1 : 200000 —	1 : 2000 —	1 : 10000 —
52	2.7.tetramethyldiaminoacridine hydrochloride (acridine orange)	1 : 200000 +	1 : 200000 +	1 : 10000 +	1 : 40000 +
		1 : 100000 —	1 : 100000 —	1 : 4000 —	1 : 20000 —
53	2.7.tetramethyldiaminoacridine methochloride	1 : 1000000 +	1 : 400000 +	1 : 40000 +	1 : 100000 +
		1 : 400000 —	1 : 100000 —	1 : 20000 —	1 : 40000 —
54	2.7.tetramethyldiaminoacridine methonitrate	1 : 1000000 +	1 : 400000 +	1 : 20000 +	1 : 100000 +
		1 : 200000 —	1 : 200000 —	1 : 10000 —	1 : 20000 —
55	2.7.tetraethyldiaminoacridine hydrochloride	1 : 200000 +	1 : 20000 +	1 : 4000 +	1 : 1000 +
		1 : 100000 —	1 : 10000 —	1 : 1000 —	? —
56	2.7.tetraethyldiaminoacridine methochloride	1 : 2000000 +	1 : 1000000 +	1 : 40000 +	1 : 40000 +
		1 : 1000000 —	1 : 400000 —	1 : 10000 —	1 : 20000 —
57	2.7.tetraethyldiaminoacridine methonitrate	1 : 2000000 +	1 : 400000 +	1 : 10000 +	1 : 20000 +
		1 : 400000 —	1 : 200000 —	1 : 4000 —	1 : 10000 —
58	2.amino-3.methylnaphthacridine hydrochloride.....	1 : 400000 +	1 : 40000 +	1 : 20000 + (*ppt.*)	1 : 2000 + (*ppt.*)
		1 : 200000 —	1 : 2000 —	1 : 10000 — (*ppt.*)	1 : 1000 — (*ppt.*)
59	2.amino-3.methylnaphthacridine methochloride ...	1 : 10000000 +	1 : 1000000 +	1 : 100000 +	1 : 1000000 +
		1 : 1000000 —	1 : 400000 —	1 : 20000 — (*ppt.*)	1 : 100000 —
60	2.amino-3.methyl-9.phenylnaphthacridine hydrochloride	1 : 2500 +	1 : 1666 + (*ppt.*)	1 : 1666 (*inh.*) (*ppt.*)	1 : 1666 + (*ppt.*)
		1 : 1666 — (*ppt.*)	? —	? —	? —
61	2.amino-3.methyl-9.phenylnaphthacridine methosulphate	? +	1 : 100000 (*inh.*)	1 : 10000 + (*ppt.*)	1 : 20000 +
		1 : 100000 —	1 : 40000 —	1 : 1000 — (*ppt.*)	1 : 4000 —
62	2.dimethylaminonaphthacridine hydrochloride	1 : 40000 +	1 : 1000 + (*ppt.*)	1 : 20000 + (*ppt.*)	1 : 1000 + (*ppt.*)
		1 : 20000 — (*ppt.*)	? —	1 : 2000 — (*ppt.*)	? —
63	2.dimethylaminonaphthacridine methochloride	1 : 1000000 (*inh.*)	1 : 400000 +	1 : 20000 +	1 : 40000 +
		1 : 400000 —	1 : 40000 —	1 : 4000 — (*ppt.*)	1 : 20000 —
64	2.7.diacetyldiaminoacridine chloroacetate	1 : 1000 +	1 : 2000 +	1 : 1000 +	1 : 2000 +
		? —	? —	? —	? —
65	2.7.diamino-9.phenylacridine carboxylic ester	1 : 40000 +	1 : 4000 +	1 : 1000 +	1 : 1000 +
		1 : 4000 —	1 : 1000 (*inh.*)	? —	? —
66	2.7.tetramethyldiamino-9.phenylacridine carboxylic ester	1 : 100000 +	1 : 8000 +	1 : 2000 +	1 : 2000 +
		1 : 8000 —	1 : 2000 —	? —	? —
67	2.7.dihydroxy-3.6.dimethyl acridine sodium salt ...	1 : 1000 +	1 : 1000 +	1 : 1000 +	1 : 1000 +
		? —	? —	? —	? —
68	2.7.dihydroxy-3.6.dimethyl acridine methochloride	1 : 2000 +	1 : 1000 +	1 : 1000 +	1 : 1000 +
		? —	? —	? —	? —

* The 1 : 1000 solution reacts acid to litmus paper.

† This result was obtained in repeated tests; but in other experiments, again, there was a wider zone of inhibition with 1 : 20000—

triaminotriphenylmethane dyes (2). The substitution of one hydrogen atom in each of the amino groups by acetyl radicals practically abolishes the antiseptic action, *e.g.*, the sterilising concentration of 2.7. diaminoacridine chloroacetate [44] for *Staphylococcus aureus* in peptone water was 1 : 100000 and in serum 1 : 200000, and for *B. coli* in peptone water 1 : 20000, and in serum 1 : 400000 ; on the other hand, with the diacetyl derivative [64] a concentration of 1 : 2000 failed in each case to sterilise.

The carboxylic esters of 2.7. diamino-9.phenylacridine [65] and of 2.7. tetramethyldiamino-9.phenylacridine [66] were so weak as to suggest a marked depressing effect of the carboxyl group on the antiseptic property.

The replacement of the amino groups by hydroxyls also led to practical abolition of antiseptic power, as is shown in the case of 2.7.dihydroxy-3.6.dimethylacridine, of which both the sodium salt [67] and the methochloride [68] were tested.

Comparative Efficiency for Staphylococcus aureus *and* B. coli.—Antiseptic potency for *Staphylococcus aureus* and *B. coli* does not invariably run parallel ; thus the lethal concentration in serum for staphylococcus is 1 : 100000, or lower in the case of 2.7.diaminoacridine hydrochloride (or sulphate) [35] and methochloride [36], and other analogous derivatives [37–46], 2.7.tetramethyldiaminoacridine hydrochloride [52], methochloride [53] and methonitrate [54], 2.7.diamino- 3.6.dimethyl acridine hydrochloride [48], and methochloride [49], 2.7.tetraethyldiaminoacridine methochloride [56] and methonitrate [57], 2.7.diamino 3.6.dimethyl-9.phenylacridine methochloride [51], 2.amino-3.methylnaphthacridine methochloride [59]. But in the case of *B. coli* only the hydrochloride, methochloride, and analogous derivatives of diaminoacridine [35–46] and of diaminodimethylacridine [48, 49], and the methochloride of 2.amino-3. methyl naphthacridine [59] reach this level of effectiveness.

Phenazine Series.

The striking feature of this series (see Table III) is the relatively poor antiseptic power exhibited by the amino compounds in serum, especially for *B. coli.* The only compounds exactly comparable with the acridine series are those of the phenazine base [69, 70], 2.7.tetramethyl diaminophenazine [80, 81], and 2.7.diamino-3.6.dimethylphenazine [86]. The enhanced effect of the metho-compounds as compared with the hydrochlorides of the same base is evident in the phenazine series ; but is not so striking as with certain of the diaminoacridine derivatives.

The relatively greater efficiency of the methochloride of 2.dimethylamino-7.amino-6.methylphenazine [84], as compared with 2.dimethylamino-7.aminophenazine [76], and of 2.7.diamino-3.6.dimethylphenazine [86] as

Table III.—Antiseptic Action of Phenazine Series in Serum.

No.	Substance.	Organism.			
		Staphylococcus aureus: medium		*B. coli*: medium	
		Peptone water.	Serum.	Peptone water.	Serum.
69	Phenazine hydrochloride		1 : 5000 +		1 : 2500 +
			? —		? —
70	Phenazine methochloride		1 : 10000 +		1 : 40000 +
			1 : 4000 —		1 : 10000 —
71	Phenazine methosulphate		1 : 10000 +		1 : 40000 +
			1 : 4000 —		1 : 10000 —
72	2.aminophenazine hydrochloride		1 : 2000 +		1 : 1000 +
			? —		? —
73	2.aminophenazine methochloride		1 : 10000 +		1 : 10000 +
			1 : 4000 —		1 : 4000 —
74	2.3.diaminophenazine hydrochloride		1 : 1000 +		1 : 1000 +
			? —		? —
75	2.3.diaminophenazine methochloride		1 : 4000 +		1 : 1000 + (*ppt.*)
			1 : 2000 —		? —
76	2.dimethylamino-7.aminophenazine methochloride		1 : 100000 +		1 : 1000 +
			1 : 2000 —		? —
77	2.dimethylamino-7.amino-6.methylphenazine hydrochloride (see methochloride No. 84)		1 : 1000 +		1 : 1000 +
			? —		? —
78	2.dimethylamino-6.methylphenazine hydrochloride		1 : 1000 +		1 : 1000 +
			? —		? —
79	2.dimethylamino-6.methylphenazine methiodide		1 : 40000 +		1 : 10000 +
			1 : 10000 —		1 : 2000 —
80	2.7.tetramethyldiaminophenazine hydrochloride		1 : 10000* +		1 : 10000* +
			? —		? —
81	2.7.tetramethyldiaminophenazine hydriodide		1 : 5000* +		1 : 5000* +
			? —		? —

The values obtained with compounds 69–83 in peptone water are omitted on account of insufficient control of the reaction.

* On account of the insolubility of these substances higher concentrations could not be tested.

Table III.—*continued.*

No.	Substance.	Organism.			
		Staphylococcus aureus: medium		*B. coli*: medium	
		Peptone water.	Serum.	Peptone water.	Serum.
82	2.aminonaphthophenazine hydrochloride†		1 : 1000 + (*ppt.*) ? —		1 : 1000 + (*ppt.*) ? —
83	2. aminonaphthophenazine methochloride		1 : 40000 + 1 : 4000 —		1 : 40000 + 1 : 20000 —
84	2.dimethylamino-7.amino-6.methylphenazine methochloride‡	1 : 400000 + 1 : 100000 —	1 : 400000 + 1 : 200000 —	1 : 1000 + ? —	1 : 100000 + 1 : 20000 —
85	2.7.diamino-6.methylphenazine methochloride	1 : 100000 + 1 : 20000 —	1 : 100000 + 1 : 20000 —	1 : 4000 + (*ppt.*) 1 : 1000 — (*ppt.*)	1 : 20000 + 1 : 4000 —
86	2.7.diamino-3.6.dimethylphenazine methochloride	1 : 1000000 (*inh.*) 1 : 200000 —	1 : 400000 (*inh.*) 1 : 100000 —	1 : 1000 + (*ppt.*) ? —	1 : 200000 + 1 : 20000 —
87	2.aminonaphtho-7.amino-6.methylphenazine methochloride	1 : 1000000 (*inh.*) 1 : 400000 —	1 : 400000 (*inh.*) 1 : 200000 —	1 : 20000 + (*ppt.*) ? —	1 : 100000 + 1 : 40000 —
88	2.methylamino-7.amino-3.6.dimethylphenazine methochloride	1 : 1000000 + 1 : 200000 —	1 : 400000 + 1 : 40000 —	1 : 2000 + 1 : 1000 —	1 : 100000 + 1 : 20000 —
89	2.dimethylamino-7.amino-3.6.dimethylphenazine methochloride (zinc chloride compound)	1 : 400000 + 1 : 40000 —	1 : 100000 + 1 : 40000 —	1 : 2000 + ? —	1 : 40000 + 1 : 20000 —
90	2.benzylamino-7.amino-3.6.dimethylphenazine methochloride (zinc chloride compound)	1 : 1000000 + 1 : 400000 —	1 : 40000 + 1 : 10000 —	1 : 10000 + 1 : 2000 —	1 : 200000 + 1 : 4000 —
91	7.amino-6 methyl-2.dimethylamino - naphthophenazine hydrochloride	1 : 40000 + 1 : 10000 —	1 : 10000 + 1 : 1000 — (*ppt.*)	1 : 1000 + (*ppt.*) ? —	1 : 40000 + ? —
92	7.amino-6.methyl-2.dimethylamino - naphthophenazine methochloride	1 : 400000 + 1 : 200000 —	1 : 40000 + 1 : 4000 —	1 : 10000 + 1 : 4000 — (*ppt.*)	1 : 10000 + 1 : 4000 —
93	N-Methyltetrahydroquinoline-2.aminophenazine methochloride (zinc chloride compound)	1 : 400000 + 1 : 100000 —	1 : 200000 + 1 : 20000 —	1 : 4000 + 1 : 2000 —	1 : 100000 + 1 : 20000 —

* On account of the insolubility of these substances higher concentrations could not be tested.

† Only partially dissolved.

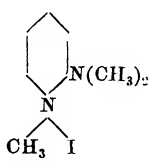
‡ The zinc chloride compound of this substance gave practically the same values.

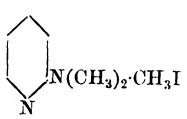
compared with 2.7.diamino-6.methylphenazine [85], suggests that methyl groups attached directly to the benzene rings may play a part in enhancing the antiseptic power in this series. For *Staphylococcus aureus* in serum the methochlorides of 2.dimethyl amino-7.amino-6.methylphenazine [84], 2.7.diamino-3.6.dimethylphenazine [86], and 2.aminonaphtho-7.amino-6.methylphenazine [87] are powerful antiseptics, practically equal to the most potent of the acridine series; on the other hand, they are markedly inferior to the latter in their action on *B. coli.*

It cannot be said that the behaviour of the phenazine series throws any clear light on the antiseptic properties of the diaminoacridine group.

Chemical Section.

Amino Pyridine Derivatives [1.2.].—*α-Amino pyridine* was obtained by the method of Tschtschibabin and Leide.* It melted at 57–58° as stated by the authors of the process. By heating with a slight excess of acetic anhydride and some fused sodium acetate it was converted into the acetyl derivative which solidified on pouring the product into water. To purify it, it was dissolved in benzene filtered from sodium acetate and the benzene removed by distillation. It melted at 72°. The acetyl derivative was warmed with methyl sulphate when the mixture heated spontaneously and on cooling solidified to a pasty crystalline mass, which was drained on a porous plate. The product, on boiling with conc. hydrochloric acid and evaporating, was not the methochloride as it contained no chlorine, but probably undecomposed methosulphate. When aminopyridine was heated in methyl alcohol solution with 4 molecules of methyl iodide in the water-bath in a sealed tube for 6—7 hours, it was converted into the dimethylamino pyridine methiodide which separated on evaporating the methyl alcohol in brown crystals of the periodide. On dissolving the periodide in water iodine separated and the methiodide passed into the solution, which is colourless. On evaporation under diminished pressure the methiodide separated in fine, colourless needles which turned yellow in the air. On analysis the compound gave 50 per cent. of iodine (calculated for $C_5H_5N(CH_3)_2CH_3I$, I = 48·1 per cent.). The constitution is, therefore, represented by one of the following formulæ:—





* 'J. S. C. I. Ann. Report,' 1915, p. 863.

[4–11.] *The Aminoquinolines and the Methochlorides.*—The *o*, *m*, *p* and *a* aminoquinolines were prepared from the corresponding nitroquinolines by reduction, the latter being obtained from the nitranilines by Knueppel's modification of Skraup's method* in which anhydrous arsenic acid is employed as oxidising agent. The *m*-nitraniline gives rise to two nitroquinolines, the *meta* and *ana* derivatives, which were separated by crystallisation from alcohol in which the *ana*-compound, m.p. 65°, dissolves more readily than the *meta*, m.p. 132-4°. In this way the meta-compound was obtained quite pure; but the ana-compound after repeated crystallisation melted at 48–50°.

The reduction of the *o*- and *p*-nitroquinolines was effected by a modification of the process described by Knueppel with iron and hydrochloric acid. The product in the case of the ortho-compound was evaporated to dryness and the residue extracted with alcohol in which the hydrochloride dissolves. The solution was made alkaline and distilled in steam. The distillate was acidified with hydrochloric acid and evaporated to dryness. The base was separated by adding caustic soda and extracting with ether. On removing the ether, the base separated in almost colourless crystals, which after recrystallisation melted at 65°. In the case of the para-compound, the product after reduction was made alkaline and extracted with ether. It melted at 108°. The *m*- and *a*-compounds were reduced with tin and hydrochloric acid. Calculated quantities of the materials were introduced into a flask and heated for a short time in the water-bath until the reaction began when the flask was removed and if necessary cooled. The tin double salt of the meta-compound, which crystallises on cooling, was separated, decomposed with excess of alkali and extracted with ether. The product, crystallised from alcohol, melted at 188–190°. In the case of the ana-compound the double salt does not separate readily and the solution was, therefore, concentrated on the water-bath. The base was extracted with ether after the addition of alkali and on crystallisation from alcohol melted at 107°. The acetyl derivatives were prepared by boiling gently for ¼ hour 1 grm. of the base with 1 grm. of fused sodium acetate and 4 c.c. of acetic anhydride. After cooling, water was added and then ammonia gradually until alkaline when the acetyl derivatives crystallised. They were recrystallised from dilute alcohol or water from which the *m*-, *p*- and *a*-compounds separated in colourless plates or flattened prisms; the *o*-compound had a faint yellow colour. The following are the melting points:—

* 'Ber.,' vol. 29, p. 703 (1896); 'Annalen,' vol. 310, p. 75 (1900).

o-Acetyl aminoquinoline		.....	103°
m-	„	„	161°
p-	„	„	138°
a-	„	„	178°

By boiling each of the above with a little conc. hydrochloric acid and concentrating the solution, the pure hydrochlorides crystallised with a brown or yellow colour and were filtered and washed with alcohol.

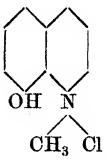
The hydrochloride of the *m*-compound dissolves in water or alcohol with a bright green fluorescence. All the hydrochlorides combine with potassium cyanate and form orange, crystalline carbamido derivatives.

The methochlorides were obtained from each of the acetyl derivatives by dissolving the substance in three to four times its weight of freshly distilled nitrobenzene and heating the solution to 150°. Rather more than the calculated quantity of dimethyl sulphate was then added and after a minute the mixture was removed from the bath. A portion of the methosulphate crystallised and the remainder was precipitated by adding ether. After standing for a time the nitrobenzene was removed as far as possible by washing with ether by decantation and then evaporating the ether by a current of air. A few cubic centimetres of conc. hydrochloric acid were added and the mixture boiled for $\frac{1}{4}$ hour. The solution was concentrated on the water-bath. The *o*- and *a*-compounds separated on cooling in brown crystalline crusts, the *m*- and *p*-compounds were crystallised from the hydrochloric acid solution by the addition of alcohol and separated in bright yellow needles. The methochloride of the meta-compound dissolves in water with a green fluorescence like the hydrochloride. The other three hydrochlorides and methochlorides exhibit no fluorescence.

[14.13] 8.*Hydroxyquinoline methopicrate and methochloride.*—These substances were prepared from the sulphate of the base (Chinosol) as follows:—

The base was separated from a solution of the sulphate by adding ammonia, filtering and washing with water. It was then dried and heated for an hour with one part of fused sodium acetate and four parts of acetic anhydride. On adding water and a little ammonia until alkaline, the acetyl derivative crystallised in long colourless needles, m.p. 75–7° after recrystallisation from dilute alcohol. The substance was then dissolved in a little toluene and an equal weight of methyl sulphate and boiled for an hour when the yellow crystalline methosulphate separated. The toluene was decanted and the dry residue boiled with conc. hydrochloric acid. In this way a reddish solution was obtained from which the methochloride did not crystallise nor was the base precipitated by ammonia. One portion was, therefore, evaporated to dryness and formed a greenish-yellow solid which is very soluble in water and

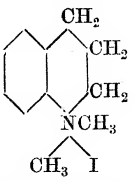
could not be obtained in the crystalline form from other solvents. To a second portion picric acid was added which precipitated the picrate in yellow clusters of microscopic needles.



8. Hydroxyquinoline methochloride.

[15.] *Tetrahydroquinoline* was prepared according to the method of Hoffmann and Königs,* by reduction with tin and hydrochloric acid. The hydrochloride crystallised from alcohol in colourless needles, m.p. 181–2°.

[16.] *Methyl tetrahydroquinoline methiodide.*—Tetrahydroquinoline obtained from the hydrochloride (2 grm.) was mixed with 14 grm. of methyl iodide at the ordinary temperature. A clear pale orange solution was obtained, from which oily drops separated and gradually solidified to a crystalline mass. The product was warmed on the water-bath with reflux for about 1 hour, and left overnight at room temperature. The excess of methyl iodide was then driven off and the yellowish crystalline mass dissolved in water and filtered. To the filtrate ammonia was added until alkaline and the methyl tetrahydroquinoline extracted with ether. The aqueous solution was evaporated nearly to dryness on the water-bath and cooled. The solid residue was pressed on a porous plate and then extracted with alcohol. On the careful addition of ether the methiodide was precipitated in colourless needles, m.p. 171–2°.



Methyl tetrahydroquinoline methiodide.

[17.] *α-Naphthoquinoline* was prepared by Knueppel's method,† applied to α-naphthylamine by Claus and Imhoff.‡ It was purified by crystallisation from petroleum ether and formed colourless needles, m.p. 45°. The metho-sulphate was prepared by dissolving the naphthoquinoline in benzene and adding an equal quantity of methyl sulphate and heating on the water-bath for a short time. Pale yellow needles of the quaternary compound separated which were filtered and dried.

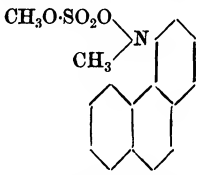
The methochloride was obtained by adding conc. hydrochloric acid and

* 'Ber.,' vol. 16, p. 728 (1883).

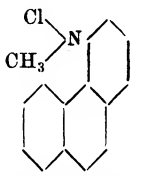
† 'Ber.,' vol. 29, p. 703 (1896).

‡ 'J. prakt. Chem.,' vol. 57, p. 68 (1898).

boiling for a short time; on adding alcohol and cooling the compound crystallised in colourless needles

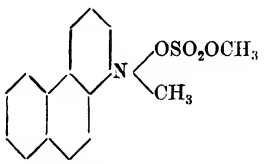


α-Naphthoquinoline methosulphate.

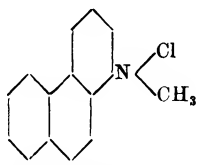


α-Naphthoquinoline methochloride.

[20. 21.] β-Naphthoquinoline was obtained by the method described by Claus and Besseler,* and purified by crystallisation from petroleum ether. It crystallises in pale yellow leaflets, m.p. 93°. It was converted into the methosulphate and chloride (as described under α-naphthoquinoline); *cf.* the following formulæ:—



β-Naphthoquinoline methosulphate.



β-Naphthoquinoline methochloride.

[22.] *Tetrahydro-α-naphthoquinoline* was obtained, as described by Bamberger,† by the reduction of α-naphthoquinoline with tin and hydrochloric acid. The base was precipitated from ethereal solution by hydrogen chloride as a crystalline mass which was purified by re-crystallisation from alcohol acidified with hydrochloric acid. After being decolourised with animal charcoal the hydrochloride of the base was obtained in colourless needles, m.p. 257°–8°.

[23.] *Tetrahydro-β-naphthoquinoline* was prepared from the β-compound, as above, in the form of colourless, lustrous leaflets, m.p. 231°.

[24.] *Diamino-β-naphthoquinoline.*—Five grms. of β-naphthoquinoline, with 6 grms. potassium nitrate and 20 c.c. conc. sulphuric acid, were heated on the water-bath for 3 hours. The nitro-compound was poured on to ice and washed with cold water. It was then warmed on the water-bath with dilute ammonia, which dissolved a brown coloured substance. The product was filtered and dried and crystallised from nitrobenzene. It melted at 230°–245° On a second crystallisation it melted at 245°–247°. On analysis,

0·16 grm. gave 21·5 c.c. moist N at 18° and 747·5 mm. N = 15·1 per cent.

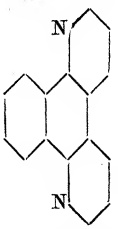
Calculated for $C_{13}H_7N(NO_2)_2$; N = 15·5 per cent.

* 'J. prakt. Chem.,' vol. 57, p. 49 (1898).

† 'Ber.,' vol. 24, p. 2475 (1891).

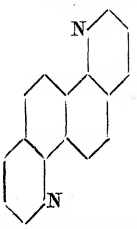
The dinitro-compound was reduced with tin and hydrochloric acid. On cooling the double stannic chloride salt crystallised and was filtered, dissolved in water, and the tin precipitated by hydrogen sulphide. From the filtrate the base was precipitated by sodium hydroxide. It formed pale yellow plates, m.p. 250°. It is very unstable and oxidises in the air.

[26.] 1.4.*Naphtho-dipyridine* (*Benzophenanthroline*) was prepared from 1.4 naphthylene diamine by Karrer's method.* The diamine was obtained by the reduction of benzene-azo-α-naphthylamine with zinc dust and acetic acid; 14·3 grm. of α-naphthylamine gave 21·6 grm. of naphthylenediamine sulphate, from which the base was obtained, m.p. 118°. The naphtho-dipyridine was purified by crystallisation from a mixture of benzene and petroleum ether and separated in long, pale yellow needles, m.p. 160°–164°.



1.4.Naphtho-dipyridine.

[27.] 1.5.*Naphtho-dipyridine.*—The 1.5.naphthylenediamine was prepared by the reduction of 1.5.dinitro-naphthalene with stannous chloride and hydrochloric acid. The naphtho-dipyridine compound was obtained, as previously described, and crystallised from benzene in nearly colourless needles, m.p. 214°–215°. It combines with methyl sulphate in hot benzene solution, from which yellow needles separated on cooling.



1.5.Naphtho-dipyridine.

[28.] 1.1.*Dinaphthyl.*2 2-*imine* was prepared by the method described by Meisenheimer and Witte† from β-naphthylamine. The latter was converted into azonaphthalene which was reduced with zinc dust and acetic acid. In this way 2.2.diamino-1.1.dinaphthyl was obtained, m.p. 191°; 2 grms. of the diamino-dinaphthyl hydrochloride were heated in the oil-bath to 240°–250°

* Marckwald, 'Annalen,' vol. 274, p. 365 (1893).

† 'Ber.,' vol. 36, p. 4154 (1903).

for 5 minutes. The cold fused mass was extracted with alcohol, poured into water, and the colourless precipitate filtered. The yield is nearly theoretical, and the product melted at 157°.

Diamino dinaphthyl. Dinaphthylimine.

Acridine Derivatives: [30] *Acridine Methochloride.*—Acridine base was precipitated from pure acridine hydrochloride by the addition of ammonia, filtered and dried: 1·5 grms. was dissolved in 7 grms. of nitrobenzene, heated to 150°, and 1·7 grms. of methyl sulphate added, and, after 1 minute, cooled. Yellow prisms of the methosulphate separated and were filtered and washed free from nitrobenzene with ether. The methosulphate is soluble in water, and from the solution the methoxide was precipitated with ammonia and crystallised from alcohol in which it is much less soluble than acridine. It crystallises in colourless plates, m.p. 140°. It dissolves readily in hydrochloric acid and the methochloride crystallises on concentration. It may be re-crystallised from alcohol and ether. It is very soluble in water, to which it imparts a green fluorescence.

[32.] 9-*Phenylacridine methochloride.*—9-Phenylacridine was prepared by the method of Bernthsen*. 50 grms. of benzoic acid and 70 grms. of diphenylamine gave 35 grms. of phenylacridine, m.p. 183°. To convert it into the methochloride the above method was adopted. The methosulphate does not separate on cooling, but on adding ether a brown oil was precipitated, which soon solidified. The ether solution was decanted and the methosulphate washed with ether. Concentrated hydrochloric acid was then added and the mixture boiled gently for a short time, when, on cooling, the methochloride separated in green leaflets, which readily dissolve in water with a yellow colour.

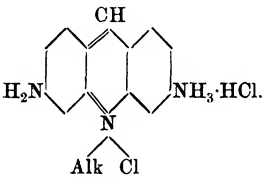
[33.] 3.6.*Dimethylacridine* was prepared according to Ullmann's method.† It crystallises from dilute alcohol in yellow needles, m.p. 171°, which fluoresce in solution with a blue colour.

The action of ethyl iodide on acriflavine in ethyl alcohol was examined with the object of obtaining the tetraethyl derivative. The mixture heated to 100° for 6 hours in a sealed tube gave a red crystalline product, which appeared to be a periodide. Its antiseptic action was inferior to that of acriflavine and it was not further investigated. It is recorded in the Table as acriflavine $+C_2H_5I$.

* 'Annalen,' vol. 224, p. 13 (1884).

† 'Ber.,' vol. 36, p. 1017 (1903).

[36–43.] 2.7.*Diaminoacridine Alkyl Chlorides.*—In order to study the relative antiseptic action of the quaternary alkyl derivatives of diaminoacridine in addition to the methochloride (acriflavine), the ethyl, propyl, *n*-butyl, isobutyl, isoamyl, phenyl, and benzyl chlorides were prepared, having the following general formula:—



The alkyl chlorides were obtained by the following method: A weighed quantity of sodium was dissolved in the alcohol corresponding to the required alkyl compound, and the theoretical amount of *p*-toluene sulphonic chloride added. After standing for a time, the product was shaken two or three times with water. The washed product was dissolved in ether, dried over calcium chloride, the ether distilled, and the residue heated *in vacuo* on the water-bath. In this way the alkyl *p*-toluene sulphonic esters were obtained in the form of pale yellow oils.

$$CH_3\cdot C_6H_4\cdot SO_2Cl + RONa = CH_3\cdot C_6H_4\cdot SO_3R + NaCl.$$

About 1 grm. of each ester was allowed to react with an equivalent weight of diacetyldiaminoacridine in nitrobenzene solution at 150°–160° The product in each case was hydrolysed with conc. hydrochloric acid, whereby the alkyl chloride was obtained.

The benzyl chloride compound, which was prepared in a similar way, was a reddish brown crystalline substance, and on analysis

0·152 grm. gave 0·132 grm. AgCl. Cl = 21·6 per cent. Calculated for $C_{20}H_{17}N_3Cl_2$; Cl = 19·2 per cent.

The phenyl chloride compound was obtained by dissolving phenol in the calculated quantity of sodium hydroxide solution to form sodium phenate. The equivalent amount of *p*-toluene sulphonic chloride was then added and the mixture boiled for about half an hour, and cooled. The phenyl *p*-toluene sulphonic ester which separated was recrystallised from alcohol and melted at 95°. It was transformed into the acridine phenochloride in the following way: About 10 per cent. more than the theoretical amount of the ester was added to the acetyl diaminoacridine dissolved in nitrobenzene and heated for 5 minutes to 150°–160°. The product was cooled to 105°, when 5 c.c. of conc. hydrochloric acid was added and the mixture maintained at 100° for another 5 minutes and cooled to the ordinary temperature. The nitro-

benzene was decanted from the phenochloride, which separated, and the latter boiled with a little more conc. hydrochloric acid, cooled, and the crystalline product filtered, washed with conc. hydrochloric acid, and finally with ether.

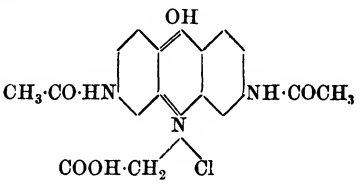
[44.] 2.7.*Diaminoacridine Chloroacetate.*—About 1 grm. of diacetyl diaminoacridine was dissolved in about 50 c.c. of nitrobenzene and heated in a metal bath to 140°–150°. An equivalent amount of chloracetic acid was added and maintained at 140°–150° for about 10 minutes. On cooling, yellow crystals separated, which were filtered and washed with ether. The substance was readily soluble in water. On analysis,

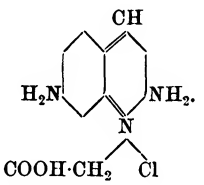
0·1485 grm. gave 0·055 grm. AgCl; Cl = 9·0 per cent. Calculated for $C_{19}H_{17}N_3O_4Cl$; Cl = 9·05 per cent.

On boiling the diacetyl derivative with conc. hydrochloric acid, red crystals were obtained, which were purified by dissolving in water and reprecipitating with conc. hydrochloric acid.

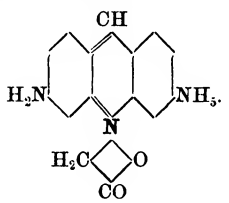
On analysis:

0·141 grm. gave 0·127 grm. AgCl; Cl = 22·2 per cent. Calculated for $C_{15}H_{13}N_3O_2Cl_2$; Cl = 20·9 per cent.





On addition of ammonia it was converted into the betaïne

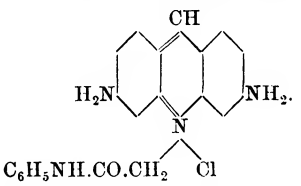


[46.] 2.7.*Diaminoacridine Chloracetanilide* was prepared by adding an equivalent of chloracetanilide to diacetyl diaminoacridine in nitrobenzene (1 grm. of the diacetyl derivative in 50° c.c. of nitrobenzene), heated to 140°–150° and maintained for 5 to 10 minutes. On cooling, an amorphous brown precipitate separated, which was filtered, washed with ether, and boiled with conc. hydrochloric acid. Brown crystals were obtained and were purified by dissolving them in water, and reprecipitating with conc. hydrochloric acid.

On analysis:

0·130 grm. gave 14·5 c.c. N at 15° and 757 mm. N = 13 per cent.
Calculated for $C_{21}H_{19}N_4OCl_2$; N = 13·4 per cent.

The compound has therefore the following formula :—



[45.] 2·7.*Diaminoacridine Chloropropionate* was prepared like the chloracetate and was purified by crystallisation from concentrated hydrochloric acid.

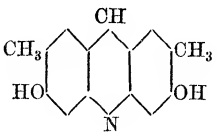
[48.] 2.7.*Diamino*-3.6.*dimethylacridine* (Acridine yellow) was prepared according to the method of Ullmann and Naef* and Ullmann and Marie.† To purify it, it was ground while moist and warmed on the water-bath with sufficient sodium hydroxide to render the mixture alkaline ; after cooling it was filtered, washed and pressed down. The moist precipitate was then dissolved in a small quantity of glacial acetic acid and whilst hot a little conc. hydrochloric added gradually until the solution changed to a deep red, when water was added till turbid and cooled. The hydrochloride separates in fine prismatic, deep orange or red crystals which dissolve in water with a green fluorescence.

[49.] 2.7.*Diamino*-3.6.*dimethylacridine methochloride.*—The hydrochloride prepared as above was precipitated as the base with sodium hydroxide, filtered, washed and thoroughly dried in a vacuum desiccator and then converted into the diacetyl derivative. 3·6 grms. of the base were mixed with 13 grms. of acetic anhydride in a small flask furnished with an air condenser and boiled gently for ¼ hour. The substance gradually dissolved. It was diluted with water, and to the cooled solution just sufficient ammonia was added to decompose the anhydride and precipitate the acetyl derivative. After filtering, washing and drying it was extracted with a little absolute alcohol, which removes a small quantity of a pale yellow substance. 1 grm. of the acetyl derivative was dissolved in 10 grms. of nitrobenzene, heated to 170° and 0·8 grm. of methyl sulphate added. On cooling, the mixture solidifies to a crystalline mass which was filtered and washed free from nitrobenzene with ether. The crystalline residue was boiled with conc. hydrochloric acid for ½ hour, when nearly the whole dissolved with a deep red

* 'Ber.,' vol. 33, p. 913 (1900).
† 'Ber.,' vol. 34, p. 4308 (1908).

colour. It was filtered and cooled. The methochloride separates in brown, glistening plates, which dissolve in water but are less soluble in dilute hydrochloric acid. It dissolves in alcohol with green fluorescence.

[67.] 2.7.*Dihydroxy*-3.6.*dimethyl acridine.*—The compound was obtained from tetramethyl diamino ditolylmethane, by the method of Ullmann and Fitzenkam*



It was converted into the diacetyl derivative by boiling with acetic anhydride and fused sodium acetate.

[68.] 2.7.*Dihydroxy*-3.6.*dimethyl acridine methochloride.*—The acetyl derivative was dissolved in toluene and to the solution an excess of methyl sulphate was added and the mixture boiled. On standing, orange needles separated and were filtered and washed with toluene. On heating the substance with conc. hydrochloric acid on the water-bath, it first dissolved and then suddenly formed a pasty mass of clusters of lemon yellow needles of the methochloride. The crystals were filtered and washed with cold water and then with alcohol. In the latter it dissolves slightly with green fluorescence. It dissolves to some extent also in hot water with a yellow colour, and is readily soluble in ammonia and sodium hydroxide solutions.

[50.] 2.7.*Diamino*-3.6.*dimethyl*-9.*phenyl acridine* (Benzoflavine) was prepared according to the method of Meyer and Gross.† It was purified by precipitating the base with ammonia, dissolving the filtered, washed and well-pressed base in glacial acetic acid, adding conc. hydrochloric acid and diluting until a permanent turbidity was formed. The hydrochloride separates in red crystals.

[51.] 2.7.*Diamino*-3.6.*dimethyl*.9-*phenyl acridine methochloride* was obtained by the method employed in the case of acridine yellow by heating the dry base with acetic anhydride and methylating the acetyl derivative with methyl sulphate. On hydrolysis of the methosulphate with hydrochloric acid the methochloride separates in bright red needles. In the absence of hydrochloric acid, it dissolves in water and on cooling gelatinises.

[52.] 2.7.*Tetramethyl diamino-acridine* (Acridine orange) was obtained by the method of Behringer‡ and Ullmann and Marie.§ Tetramethyl-diamino

* 'Ber.,' vol. 38, p. 3787 (1905).
† 'Ber.,' vol. 32, p. 2356 (1899).
‡ 'J. prakt. Chem.,' vol. 54, p. 240 (1896).
§ 'Ber.,' vol. 34, p. 4307 (1901).

diphenylmethane, prepared by the action of formaldehyde on dimethyl aniline in presence of dilute sulphuric acid was nitrated in the cold with potassium nitrate and conc. sulphuric acid and the purified dinitro-derivate, m.p. 141°–2°, reduced with stannous chloride. After reduction the base m.p. 141°–2° was precipitated with caustic soda and extracted from the stannous hydroxide with alcohol. It was converted into the acridine compound m.p. 181°–2° as described under tetraethyldiamino acridine (see below) and the latter into the methosulphate. From the solution of the methosulphate potassium nitrate precipitates the methonitrate in red needles from which the methoxide was obtained as a hygroscopic mass on the addition of ammonia. The methochloride is too soluble and hygroscopic to be separated, and closely resembles the corresponding ethyl derivative described below.

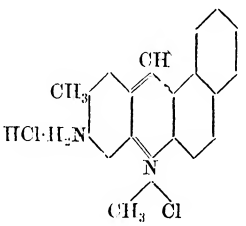
[55.] 2.7.*Tetraethyldiamino-acridine* was prepared by the above method using diethylaniline in place of dimethylaniline. The tetraethyldiamino diphenyl methane is a colourless liquid, which boils at 285°–290° at 10 mm. and 305°–310° at 20 mm. pressure. The yield from 100 grms. of diethyl-aniline was 50 grm. of the diphenylmethane derivative. On nitration it formed a dinitro derivative which crystallised from acetic acid in orange red plates m.p. 118°–119°. The latter was reduced with tin and hydrochloric acid and the tin partly removed by hydrogen sulphide. The filtered solution on evaporation left a colourless, resinous mass of the hydrochloride which contained some tin salt. 2 grms. of the hydrochloride were heated with 4 c.c. of conc. hydrochloric acid diluted with 12 c.c. of water at 140° for 6 hours in a sealed tube. The red coloured contents of the tube were extracted with boiling water in which they dissolved leaving very little residue, and to the hot solution ferric chloride solution was added until no more precipitate was formed. After the addition of a solution of common salt, which throws down a further quantity of the acridine compound, the mixture was filtered after cooling and washed with salt solution. The deep red solution was made alkaline with ammonia which precipitates the yellow base. The latter was filtered and washed and dissolved in hydrochloric acid. On concentrating the solution, the hydrochloride crystallises in red crusts with a green iridescence. The crystals dissolve in water with a bright orange colour.

[56. 57.] 2.7.*Tetraethyldiamino-acridine methochloride and methonitrate.*—To prepare the methonitrate an excess of dimethyl sulphate was added to the finely powdered base in a small basin. The mixture becomes hot. After heating for a time on the water-bath, the methosulphate crystallises. Water was then added, and heating continued until the excess of methyl sulphate was decomposed and a clear red solution obtained. On the addition of a

saturated solution of potassium nitrate, the methonitrate crystallised on standing in red needles. They were filtered, washed with a little water, and dried. On grinding with ammonia solution the methoxide was obtained as a sticky red mass with green iridescence which dissolves in hydrochloric acid forming the methochloride as a hygroscopic mass which could not be prepared in the crystalline state.

[58.] 2.*Amino*-3.*methylnaphthacridine* was prepared according to the directions of Ullmann and Naef.* The base, after re-crystallisation from xylene, melts at 244°. It was acetylated, as described, and the acetyl derivative melted at 320°–321°.

[59.] 2.*Amino*-3.*methylnaphthacridine methochloride*.—0·6 grm. of the acetyl derivative was dissolved in 2 c.c. of nitrobenzene heated to 160°, and 0·3 grm. of dimethyl sulphate added when the mixture crystallised to a semi-solid mass, which was filtered and washed with ether. The product, after boiling with conc. hydrochloric acid, became dark red, and on concentrating the solution on the water-bath and cooling the methochloride crystallised in long red needles.



[60.] 2.*Amino*-3.*methyl*-9.*phenylnaphthacridine*. — The substance was prepared by adding 5 grms. of tetramino-ditolylphenyl-methane (Meyer and Gross),† to 8 grms. of β-naphthol at 150° and heating for an hour to 180°–200°. The sticky product was dissolved in 10 c.c. of glacial acetic acid, diluted with an equal volume of water, and poured into 10 grm. of caustic soda in 50 c.c. of water. The yellow precipitate was filtered and washed with dilute caustic soda solution and hot water to remove the naphthol, and the product crystallised from alcohol. The undissolved portion was filtered off. It crystallises from nitrobenzene and also from benzene in yellowish brown needles, m.p. 264°–265°. The alcoholic filtrate was heated to boiling, water added till turbid, and allowed to cool. The crystalline product was re-crystallised from benzene from which it separates in yellow needles, m.p. 269°–271°.

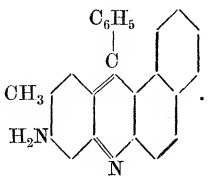
* 'Ber.,' vol. 33, pp. 915, 2473 (1900).
† 'Ber.,' vol. 32, p. 2356 (1899).

The compound of m.p. 264°–265° on analysis

0·2026 grm. gave 14·3 c.c. moist N at 14° and 725 mm. N = 8·37 per cent.

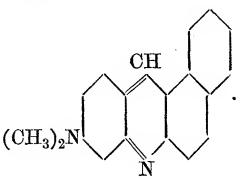
0·1960 grm. gave 13·7 c.c. moist N at 16° and 753 mm. N = 8·24 per cent. Calculated for $C_{24}H_{18}N_2$; N = 8·4 per cent.

The compound has therefore the formula:



It was converted into the methosulphate by dissolving in eight times the weight of nitrobenzene at 130° and adding half the weight of methylsulphate. The crystalline product was filtered and washed with ether.

[62. 63.] 2.*Dimethylaminonaphthacridine and methochloride.*—

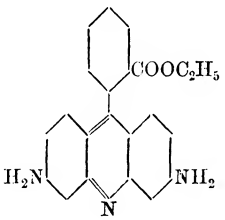


It was prepared by the method of Ullmann and Marie,* by fusing tetramethyldiaminodiphenyl methane with β-naphthol at 110°–120°, and raising the temperature gradually to 180°–200°. The product was extracted with warm alcohol which dissolves the naphthacridine compound, but leaves the hydracridine. The hydracridine compound was suspended in boiling alcohol, acidified with a few drops of hydrochloric acid and ferric chloride added until the precipitation of the naphthacridine hydrochloride was complete. The precipitate was filtered, dissolved in water, and re-precipitated with conc. hydrochloric acid. It was filtered and dried *in vacuo* over caustic soda. The base was precipitated from the dissolved hydrochloride with ammonia, and after re-crystallisation from benzene melted at 185°. It was dissolved in boiling xylene and the calculated amount of methyl sulphate added. The precipitated methosulphate was washed with ether and dried; on dissolving it in water and adding sodium chloride solution the methochloride was precipitated, filtered, and dried. To remove the sodium chloride the methochloride was dissolved in alcohol, filtered, and evaporated to dryness. The naphthacridine base was obtained from the first alcoholic filtrate by adding a

* 'Ber.,' vol. 34, p. 4318 (1901).

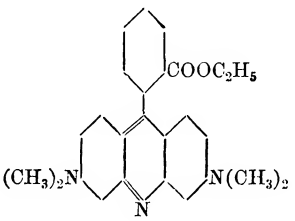
boiling alcoholic solution of picric acid. The picrate which crystallised was filtered and re-crystallised from boiling aniline. To the picrate suspended in alcohol, caustic soda was added and warmed on the water-bath until the solution was deep yellow, and then diluted. The precipitated base was filtered, washed with water, dried and re-crystallised from benzene (m.p. 185°).

[65.] 2.7.*Diamino*-9.*phenylacridine carboxylic ester.*—



The substance was prepared by heating with ammonia in sealed tubes according to the method of Meyer and Oppelt.* The product was boiled with alcohol to remove impurities and the residue suspended in alcohol and hydrogen chloride passed in. The alcohol was removed, the hydrochloride dissolved in water and precipitated with salt, redissolved and again salted out. The product is an orange amorphous powder.

[66.] 2.7.*Tetramethyldiamino*-9.*phenylacridine carboxylic ester.*



One molecular proportion of phthalic anhydride was heated with three of acetic anhydride and two of *m*-aminodimethylaniline for 2–3 hours at 140°–150°. The acetic acid was then distilled off and the residue boiled with fifteen to twenty parts of 20 per cent. hydrochloric acid for ½ hour. From the deep red solution the base was precipitated by ammonia, filtered and dried in vacuo. It was suspended in ten times its weight of absolute alcohol, heated an hour with reflux on the water-bath whilst dry hydrogen chloride was passed in. The alcohol was removed, the residue dissolved in hot water, filtered and the filtrate salted out. The precipitate was filtered, washed with a little cold water and dried.

* 'Ber.,' vol. 21, p. 3376 (1888).

PHENAZINE DERIVATIVES.

[69–71.] Phenazine was prepared by the method of Kehrmann and Havas* by heating a mixture of *o*-amino- and *o*-nitro-diphenylamine with fused sodium acetate. It melts at 170°—171°. The methosulphate and methochloride were obtained in the same manner as the corresponding acridine compounds. The phenazine was dissolved in five times its weight of nitrobenzene, heated to 120°, and freshly distilled methyl sulphate equal in weight to the phenazine was added, the mixture stirred and kept for 5 minutes at 100°–110° and cooled. The methosulphate separates; ether was added to complete the precipitation and the product filtered and washed. It forms greenish yellow prisms. The methochloride was prepared by first separating the base with ammonia and evaporating the red solution, taking up with alcohol to remove ammonium sulphate and then evaporating to dryness. The methochloride was prepared by dissolving the base in hydrochloric acid and concentrating the solution. It forms greenish crystals.

[72.] 2.*Aminophenazine* was prepared by (A) the method described by Wohl and Lange,† from *o*-nitraniline and aniline-hydrochloride in presence of fused zinc chloride. The amino-phenazine was separated from the product by sublimation and crystallises in brilliant red needles melting at 283°, which after crystallisation melt at 288°. The acetyl derivative was obtained by heating for a short time with an equal weight of fused sodium acetate, and twelve to fifteen times its weight of acetic anhydride. When poured into water and neutralised with ammonia it formed a buff-coloured crystalline powder. The dry acetyl derivative was dissolved in ten times its weight of nitrobenzene heated to 120° and methyl sulphate, equal in weight to the acetyl derivative, added and the mixture cooled slowly. Ether precipitates the methosulphate, which was filtered and washed with ether. It was then boiled with water to remove nitrobenzene and filtered. About an equal volume of conc. hydrochloric acid was added and evaporated to dryness. The methochloride, which is very soluble, was dissolved in alcohol, filtered and evaporated. It forms a red, crystalline residue which dissolves in water and alcohol with a bright magenta colour.

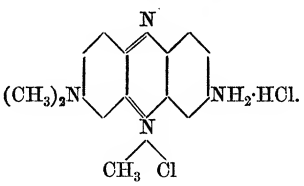
(B) A second process for preparing aminophenazine is to pass dry ammonia into an alcoholic solution of phenazine methyl sulphate according to the method of Kehrmann and Havas.‡ The products in the two cases appeared to be identical and to have identical bactericidal properties.

[74.] 2.3.*Diaminophenazine* was prepared by the method of Ullmann

* 'Ber.,' vol. 46, p. 341 (1913).
† 'Ber.,' vol. 43, p. 2186 (1910).
‡ 'Ber.,' vol. 46, p. 431 (1913).

and Mauther,* and converted into the acetyl derivative.† It was recrystallised from nitrobenzene and forms a light brown micro-crystalline powder which turns brown at 206° and melts about 270°. The latter was dissolved in ten times its weight of nitrobenzene at 150°, and 1 mol. of methyl sulphate added. The methosulphate, which was precipitated, was washed with ether and the product boiled with conc. hydrochloric acid and evaporated on the water-bath. The methochloride crystallised in black needles which were filtered and dried.

[76.] 2.*Dimethylamino*-7.*aminophenazine methochloride.* — The compound was prepared according to the method described by Karrer,‡ from a mixture of para- and meta-dimethyl phenylene diamine by oxidation with potassium dichromate. The phenazine salt was precipitated by zinc and sodium chloride and the base separated from the solution in hydrochloric acid by sodium hydroxide. It was purified by redissolving in acetic acid and precipitating with ammonia. It forms a blue-black powder slightly soluble in alcohol with a deep violet colour, and readily soluble in dilute acids giving a violet solution and forming the hydrochloride



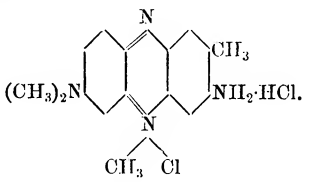
[77.] 2.*Dimethylamino*-7.*amino*-6.*methylphenazine hydrochloride* (*Toluylene red*).—The substance was obtained by following the directions of Witt.§ It was purified by crystallisation from alcohol. The base was obtained by precipitation with sodium hydroxide, and was washed, dried and acetylated in the usual way. The acetyl derivative crystallises in brown plates. The methiodide was obtained in microscopic black crystals which dissolve in water and alcohol with a deep violet colour, by heating the acetyl derivative with methyl iodide in a sealed tube for several hours to 100°.

[84.] 2.*Dimethylamino*-7-*amino*-6.*methylphenazine methochloride.* — The above acetyl derivative was dissolved in five times its weight of nitrobenzene heated to 160°, and rather more than the calculated weight of methyl sulphate added. After a minute or two the mixture was cooled and the resulting black precipitate washed with ether and dried. The product was then hydrolysed with conc. hydrochloric acid, when the liquid became deep

* 'Ber.,' vol. 35, p. 4302 (1902).
† Fischer and Hepp, 'Ber.,' vol. 22, p. 358 (1889).
‡ 'Ber.,' vol. 49, p. 1643 (1916); vol. 50, p. 420 (1917).
§ 'Ber.,' vol. 12, p. 921 (1879).

blue. On adding water or salt solution, the methochloride separated in the form of a semi-solid iridescent green mass, which after drying became hard and was crystallised from a mixture of benzene and alcohol in the form of a micro-crystalline powder with a dark green lustre. It dissolves in water and alcohol with a bright magenta colour, similar to toluylene red. On analysis, found N = 17·0 per cent.; $CH_{20}N_4Cl_2$ requires N = 16·5 per cent.

The formula is therefore



Another method for the preparation is given in D.R. Patent 69188.* It consists in heating together 3 mols. *p*-nitrosodimethylaniline hydrochloride and 2 mols. *o*-aminodimethyl-*p*-toluidine in 50 per cent. acetic acid solution for 6 hours. On dilution, filtration, and precipitation with common salt, a green crystalline colouring matter was precipitated, which was filtered and, on account of its solubility in water, washed with brine. To the mother liquors, which still contained a quantity of the dye zinc chloride was added, and the zinc chloride double salt precipitated which was re-crystallised from dilute hydrochloric acid. The substance dissolved with a distinctly magenta colour. There appears, therefore, to be a graduation in tint from scarlet to magenta with the increase of alkyl radicals in the amino group (compare [86] and [88]). The same development of blue in the colour is observed in rosaniline and its methyl derivatives which change from magenta to violet.

Bromination of Acetyl Derivative of Toluylene Red.—The acetyl derivative was brominated in chloroform solution, when a dark violet solution was formed, which on evaporation gave a dark violet product, which crystallised from a mixture of alcohol and ether in microscopic black prisms. The substance is, however, a mixture, which can be separated by extracting with ethyl acetate. The undissolved portion was dissolved in water and the base precipitated with ammonia as an oil which solidified on standing. It crystallises from alcohol in pale brown transparent prisms, m.p. 205°–207°. It was tested qualitatively for bromine, but its further investigation was postponed.

[78.] 2.*Dimethylamino*-6.*methylphenazine.*—Toluylene red was diazotised according to Witt's method (*loc. cit.*) in absolute alcohol with sodium nitrite and hydrochloric acid. The excess of alcohol was then removed on the water-bath, the liquid filtered and poured into a solution of sodium acetate,

* 'Friedländer,' vol. 3, p. 397.

which throws down the phenazine compound in the form of a crystalline precipitate having a bronzy iridescence.

It was recrystallised from dilute alcohol and melted at 168°–169°. The ether and benzene solutions are fluorescent, but fluoresce with different colours, the former having an orange and the latter a greenish colour.

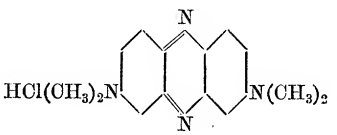
[79.] 2.*Dimethylamino*-6.*methylphenazine methiodide* was prepared from the phenazine compound by heating with rather more than the calculated quantity of methyl iodide in a sealed tube to 100° for 6 hours. A solid dark violet mass resulted, which dissolved in water with a deep violet colour. The substance was recrystallised from a mixture of alcohol and ether, from which it separated in brownish black plates. On analysis,

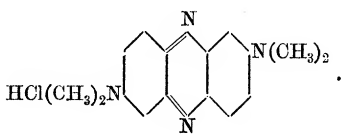
0·2525 grm. gave 0·1718 grm. AgI; I = 36·8 per cent.

0·2540 grm. gave 0·1714 grm. AgI; I = 37·0 per cent. $C_{15}H_{15}N_3+CH_3I$ requires I = 33·5 per cent.

The iodine value found is too high, the reason for which is not clear unless, as often happens in such cases, a certain amount of periodide is formed at the same time.

[80, 81.] 2.7.*Tetramethyldiaminophenazine* is described by Karrer* as being obtained by oxidising a mixture of dimethyl-*p*-phenylenediamine and dimethyl-*m*-phenylenediamine with potassium dichromate solution in presence of hydrochloric acid. The product may have either of the following formulæ:—





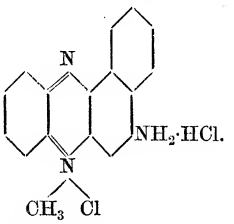
It was separated in the form of the hydriodide, from which the base was precipitated with ammonia, filtered, washed, and extracted with alcohol. The residue was dissolved in dilute hydrochloric acid and the solution was evaporated in a vacuum desiccator to dryness. The methochloride was prepared in the usual way by the action of methyl sulphate on the base, dissolved in nitrobenzene and subsequent hydrolysis with conc. hydrochloric acid. It dissolves in water with a bluish red colour.

[82, 83.] 2. *Aminonaphthophenazine* was prepared, according to the method described by O. Fischer and Hepp,† by heating in a sealed tube for 5 to 6 hours at 160° one molecule *o*-phenylenediamine and one molecule benzene-azo-*α*-naphthylamine hydrochloride with ten parts of alcohol. On cooling, dark red

* 'Ber.,' vol. 49, p. 1643 (1916).

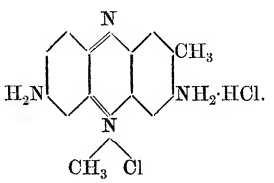
† 'Ber.,' vol. 23, p. 845 (1890).

crystals separated. They were filtered and crystallised from alcohol containing hydrochloric acid. The substance obtained in this way is the hydrochloride of aminonaphthophenazine. It forms dark red crystals, which are slightly soluble in water, but on heating are hydrolysed and the base is precipitated. The acetyl derivative was prepared by heating the base with acetic anhydride and fused sodium acetate. On cooling, the acetyl derivative crystallises. It was heated to 160° with ten to twenty times its weight of nitrobenzene and the equivalent of one molecule of methyl sulphate added. On standing, crystals of the metho-sulphate separated. They were filtered and washed with ether and heated with conc. hydrochloric acid, when the hydrochloride of the methochloride slowly crystallised. The substance is soluble in water and not so readily hydrolysed as the hydrochloride.



2. Amino-naphtho phenazine methochloride.

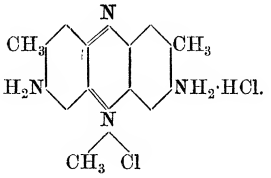
[85.] 2.7.*Diamino*-6.*methylphenazine methochloride* was prepared according to D.R. Patent 86608* by heating together and stirring amino-azobenzene hydrochloride, amino dimethyl-*p*-toluidine and glycerol at 110°, according to the proportionate amounts given. The mixture froths up during the process, and the reaction is at an end when frothing ceases (about 3 hours). The solid product was dissolved in hot water and precipitated as hydrochloride by the addition of hydrochloric acid and some common salt. The colouring matter was re-crystallised from dilute hydrochloric acid, giving green, glistening crystals, which dissolved in water with a scarlet colour.



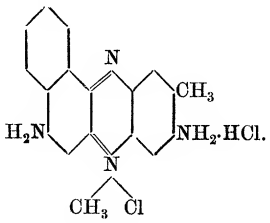
[86.] 2.7.*Diamino*-3.6.*dimethylphenazine methochloride* was prepared as above, using *o*-amino azotoluene hydrochloride in place of the benzene derivative. The product was dissolved in hot water and precipitated with hydrochloric acid. On cooling the phenazine compound separated in green prismatic

* 'Friedländer,' vol. 4, p. 380.

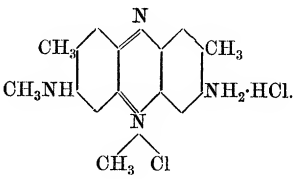
needles, very slightly soluble in cold water, soluble in hot water with a scarlet colour.



[87.] 2.*Aminonaphtho*-7.*amino*-6.*methylphenazine methochloride.*—The preparation was carried out as above, using proportionate quantities of benzene-azonaphthylamine hydrochloride and one-and-a-half times the amount of glycerol, and heating at 120° for 3½ hours. The hot aqueous solution of the product was filtered from insoluble matter, and the colouring matter precipitated with hydrochloric acid and salt. It was re-crystallised from dilute hydrochloric acid from which it separated in small green crystals. It dissolves in water with a scarlet colour similar in tint to the other two.



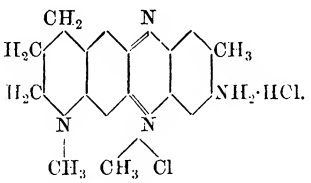
[88.] 2.*Methylamino*-7.*amino*-3.6.*dimethylphenazine methochloride.*—The substance was prepared from nitrosomethylaniline hydrochloride and *o*-amino-*p*-dimethyl toluidine dissolved in alcohol according to the quantities given in D.R. Patent 80758.* The mixture was boiled for 6 hours. The scarlet colour developed in a few minutes and intensified rapidly, until signs of precipitation appeared when the liquid was somewhat concentrated and cooled. The precipitate was filtered and washed with a little alcohol. The phenazine compound consists of a green crystalline powder which dissolves in water with a more magenta colour than the previous compound.



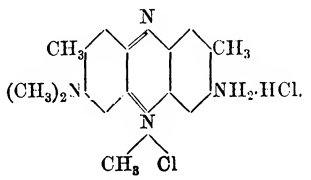
[93.] *N-Methyltetrahydroquinoline*-2.*aminophenazine methochloride.* — The tetrahydroquinoline and its N-methyl derivative were prepared by the

* 'Friedländer,' vol. 4, p. 376.

method of Hoffman and Königs,* and converted into the nitroso compound.† A mixture of 2·6 grm. nitroso N-methyl tetrahydroquinoline, 1·5 grm. of *p*-dimethylamino *o*-toluidine, 15 c.c. of glacial acetic acid and 2 c.c. of conc. hydrochloric acid were warmed gently on the water-bath. Heat was developed and the liquid became a brilliant green which rapidly changed through brown black, dull scarlet to magenta. The heating was continued from 3 to 4 hours, when the product was dissolved in 50 c.c. of hot water, filtered and the colouring matter precipitated with zinc chloride and brine. It forms a green iridescent mass which is at first sticky, but rapidly hardens. The substance dissolves in water with a magenta colour similar to that of toluylene red methochloride (p. 360). On analysis the zinc salt gave N = 14·5 per cent.; $C_{36}H_{42}N_8Cl_4Zn$ requires N = 14·1 per cent. The formula of the hydrochloride is therefore



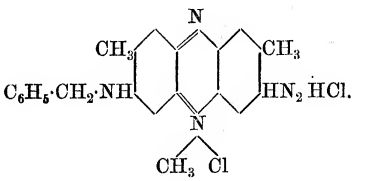
[89.] 2.*Dimethylamino*-7.*amino*-3.6.*dimethylphenazine methochloride.* — 16 parts of *p*-amino-dimethyl *o*-toluidine and 15 parts of *m*-dimethylamino *p*-toluidine were dissolved in 300 parts of water, 20 parts of conc. hydrochloric acid and 30 parts of 50 per cent. acetic acid, and cooled to 10° with stirring. Thirty parts of sodium dichromate in 400 parts of water were run in during 3 hours. A bluish-red coloration develops immediately and becomes more intense as the oxidising agent is added. The mixture was finally warmed on the water-bath for an hour, filtered and salted out with zinc chloride and sodium chloride. In this way the zinc chloride salt of the colouring matter is obtained. It was purified by solution in water, filtration and reprecipitation with salt solution. It could not be recrystallised from alcohol or dilute hydrochloric acid. The substance by analogy with the formation of tetramethyldiamino-phenazine (p. 361) has the following structure :—



* 'Ber.,' vol. 16, p. 728 (1883).

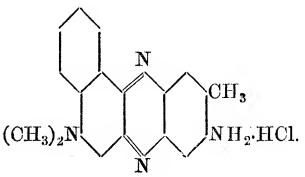
† Königs and Freer, 'Ber.,' vol. 18, p. 2388 (1885).

[90.] 2.*Benzylamino*-7.*amino*-3.6.*dimethylphenazine methochloride.* — The compound was prepared by heating together on the water-bath *p*-dimethylamino *o*-toluidine dissolved in alcohol with nitroso benzyl *o*-toluidine hydrochloride and hydrochloric acid as described in the preparation of the methylamino compound (p. 361). The colour developed passing from yellowish red to red, with a faintly blue tint. On concentration and cooling the colouring matter separated and was filtered and washed with ether. It has probably the following formula:—



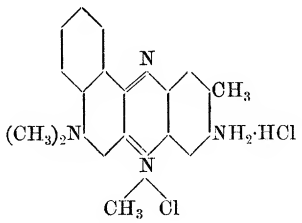
The zinc salt is obtained by precipitating the methochloride in solution with zinc chloride.

[91.] 7.*Amino*-6.*methyl*-2.*dimethylamino-napthophenazine hydrochloride.*— The substance was prepared as follows: 2·3 grms. of nitroso dimethyl α-naphthylamine hydrochloride were dissolved in 20 c.c. of hot glacial acetic acid, and to the solution was added a solution of 1·2 grms. of *m*-toluylene diamine in 20 c.c. of 50 per cent. acetic acid. 1 c.c. of conc. hydrochloric acid was then added and the mixture heated on the water-bath for 4 hours. A magenta colour developed rapidly and lost its bluish tint on continued heating, becoming gradually more crimson. It was finally boiled, diluted with 100 c.c. of water and the hydrochloride precipitated with brine. For purification it was redissolved in water and reprecipitated with brine. It has the following formula:—



[92.] 7.*Amino*-6.*methyl*-2.*dimethylaminenapthophenazine methochloride.* — The process was carried out as above, but instead of *m*-toluylene diamine the *p*-dimethylamino *o*-toluidine was used. 2·4 grms. of nitroso dimethyl α-naphthylamine hydrochloride were dissolved in 20 c.c. glacial acetic acid and added to 1·5 grms. of dimethylamino-toluidine in 20 c.c. 50 per cent. acetic acid, and 1 c.c. of conc. hydrochloric acid. The bright crimson colour develops rapidly, and after 3 hours' heating on the water-bath was separated

and purified as in the previous case. The hydrochloride of the methochloride was even less soluble than the above hydrochloride, but is readily soluble in alcohol. The formula is



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4. Browning and Gulbransen, 'Roy. Soc. Proc.,' B, vol. 90, p. 136 (1918).
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